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Neurobiological substrates of cognitive rigidity and autonomic inflexibility in generalized anxiety disorder

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Highlights

- Infrequent targets detection mainly deactivated default mode brain regions.
- Trait worry was inversely related with prefrontal deactivation to target detection.
- HRV predicted neural activation changes produced by attentional focus switching.
- A worry induction increased pre- to post-target deactivation of default mode areas.
- The induction reduced behavioural and neurobiological differences between groups.

Abstract

Generalized anxiety disorder (GAD) is characterized by difficulties in inhibiting both perseverative thoughts (worry and rumination) and autonomic arousal. We investigated the neurobiological substrates of such abnormal inhibitory processes, hypothesizing aberrant functional coupling within ‘default mode’ (DMN) and autonomic brain networks. Functional imaging and heart rate variability (HRV) data were acquired from GAD patients and controls during performance of three tracking tasks interspersed with a perseverative cognition (PC) induction. After detection of infrequent target stimuli, activity within putative DMN hubs was suppressed, consistent with a redirection of attentional resources from internal to external focus. This magnitude of activity change was attenuated in patients and individuals with higher trait PC, but was predicted by individual differences in HRV. Following the induction of PC in controls, this pattern of neural reactivity became closer to that of GAD patients. Results support, at a neural level, the association between cognitive inflexibility and autonomic rigidity.

Key Words: Generalized Anxiety Disorder, Perseverative Cognition, Heart Rate Variability, FMRI, Attention

Introduction

In their “autonomic flexibility-neurovisceral integration model of anxiety”, Friedman and Thayer (1998) view anxiety as a systemic inflexibility, grounded in poor inhibition. Indeed, anxious individuals show a reduced capacity to inhibit cognitive (worry), behavioural (avoidance), and accompanying physiological (reduced vagal tone) manifestations. According to this model, vagal tone, indexed by heart rate variability (HRV) may serve as a measure of the integrity of brain networks that regulate inhibition through central-autonomic nervous control in the service of efficient functioning (Friedman, 2007).

An earlier laboratory study applied this model to perseverative cognition (i.e., worry and rumination) in healthy individuals, hypothesizing a link between cognitive inflexibility and autonomic rigidity expressed as reduced HRV (Ottaviani, Shapiro, & Couyoumdjian, 2013). The rationale for collapsing worrisome and ruminative thoughts into a single phenomenal category is corroborated by studies showing no differences between these two processes on their impact on appraisals and strategies (e.g., Segerstrom, Tsao, Alden, & Craske, 2000; Watkins, Moulds, & Mackintosh, 2005) and by the incremental benefits of using perseverative cognition as a transdiagnostic symptom (McEvoy, Watson, Watkins, & Nathan, 2013; Spinhoven, Drost, van Hemert, & Penninx, 2015). In Ottaviani et al. (2013), participants performed a low-demanding tracking task before and after a perseverative cognition induction in which they were asked to recall a past or future personally relevant negative event. The task required participants to keep the cursor inside a white circle in motion on a black screen and press the left mouse button as fast as possible each time the circle turned red. Reaction times were used as an objective index of cognitive rigidity. At different time intervals, probes interrupted the task to inquire about subjects' moods and thoughts. Whenever subjects reported worrying about a future event, or ruminating about a past stressful event, they were asked how much they experienced the thought as intrusive and how much they were trying to suppress it (subjective measures of cognitive rigidity). HRV was monitored throughout the task as an index of autonomic flexibility. Worry and rumination were

expressed along attentional (slower reaction times), cognitive (efforts to inhibit intrusive thoughts), affective (mood worsening), and autonomic (lower HRV) dimensions. In line with Friedman and Thayer's model (1998), the cognitive inflexibility that characterized perseverative cognition was mirrored, at a physiological level, by autonomic rigidity. Two 24-hours ambulatory studies replicate these findings in healthy (Ottaviani et al., 2015a) and depressed participants (Ottaviani et al., 2015b).

The present study aimed to extend these results by defining neural substrates of the cognitive rigidity that characterizes perseverative cognition using the same task and induction procedure in an fMRI environment with simultaneous cardiac monitoring. HRV was derived to test, at a neural level, the association with autonomic rigidity. Importantly, we examined these effects in both healthy participants and patients with GAD, a clinical population that has perseverative cognition as a core symptom. The simultaneous assessment of bodily reactions and cognitive processes is particularly needed when studying generalized anxiety, where changes in autonomic nervous function, such as palpitations or sweating, can drive negative cognitions creating a vicious cycle that plays a major role in the maintenance of the disorder.

The integration of cognitive and affective neuroscience with clinical autonomic research has advanced our understanding of the neurobiology of GAD and related anxiety disorders. Anxiety is associated with aberrant (often exaggerated) functional activation of brain regions normally engaged in response to motivational salient stimuli and demanding behavioural challenges. These same brain regions are also implicated in the generation (e.g. anterior cingulate cortex), representation (e.g. insular cortex) or both (e.g. amygdala) of autonomic states of arousal (Critchley, Eccles, & Garfinkel, 2013). These studies are informed by observations in patients with clinical perturbations in autonomic response (e.g., Critchley, Mathias, & Dolan, 2002) and recent studies illustrating how neural responses to feedback from the heart can dynamically shape the perception and processing of threat and safety (reviewed in Garfinkel & Critchley, 2016).

Unfortunately, no published studies examining perseverative cognition in GAD have combined

neuroimaging techniques with concurrent measures of peripheral autonomic nervous activity. In fact, to our knowledge, only two published studies directly investigated the brain correlates of worry in GAD patients. One of them had a small sample size ($n = 6$ patients) and the absence of a comparison group as strong limitations (Hoehn-Saric, Schlund, & Wong, 2004). Although difficult to generalize, patients showed enhanced activation of frontal and anterior cingulate (ACC) cortices in response to worrisome sentences (Hoehn-Saric et al., 2004). The second suggested that worry induction engaged the same neural system in both GAD and healthy individuals (Paulesu et al., 2010). Activity within ACC and dorsal medial prefrontal cortex (DMPFC) was enhanced by worry inducing sentences in both GAD patients and controls but, interestingly, GAD patients also showed a sustained activation of these areas during resting state scans that followed the worrying induction phase. In line with a dimensional view of psychopathology, this persistent activation correlated with dispositional tendencies to worry assessed by the Penn State Worry Questionnaire (PSWQ; Meyer, Miller, Metzger, & Borkovec, 1990).

Overall there is need for greater neurobiological understanding of difficulties in inhibiting perseverative thoughts and autonomic dysregulation in GAD, perceived as uncontrollable and disruptive to patients' daily life. We therefore undertook a neuroimaging study, acquiring fMRI and concomitant HRV data from GAD patients and healthy controls. Participants performed repetitions of a low-demand tracking task. This task promoted occurrence of self-generated thoughts, and the degree to which these thoughts distracted participants from the task could be measured by examining pre- to post-infrequent targets brain activation patterns.

'Spontaneous' (i.e. task-free) brain activity in functional imaging experiments is no longer simply viewed as noise or a confound to be controlled (e.g., Binder et al., 1999). This motivated our analytical approach: periods of low cognitive, emotional, or sensory demand (as in our pre-target epochs), reliably enhance activity across a specific network of regions proposed to support the brain's default mode (Raichle et al., 2001). The key emergent property of DMN activation is the production of self-generated thoughts or mind wandering (Mason et al., 2007). When a task

requires our attention, however, the activation of such network is suppressed (as in our post-target epochs). Deficits in DMN suppression are reported in several mental illnesses, notably anxiety disorders (Anticevic et al., 2012). This observation suggests that difficulties in switching from rest to task are linked to specific symptomatology, e.g. impaired attention and worry (Forster et al., 2015). In line with this approach, instead of looking at what happens while participants perform a task, it can be more informative to compare periods of resting activity before and after infrequent target occurrence. The concept of task-rest interactions acknowledges the modulation of resting brain activation by the preceding stimulus-induced activation or vice-versa (Northoff et al., 2010). Thus, 15 s periods immediately preceding experience sampling probes were used to demonstrate activation of DMN regions during mind wandering (Christoff et al. 2009). Post-stimulus alteration in neural activity is dissociable from stimuli-evoked neural responses (Mullinger et al., 2013).

Between repetitions of the task, we included an induction of perseverative cognition to increase the likelihood of worry and rumination. From here on, the use of “self-generated thoughts” will indicate thoughts that are unrelated to the immediate external environment (i.e., the ongoing task) and are internally-driven (Andrews-Hanna et al., 2014). Self-generated thoughts can take the form of perseverative cognition in GAD or –in healthy individuals– after an experimental induction. The term “attentional control” will be conversely used to indicate moments in which participants’ attention is on the task (i.e., externally-oriented).

At a behavioural level, we expected to replicate previous findings (Ottaviani et al., 2013) showing that reaction times are delayed and HRV is reduced following induction, due to an increase in rumination and worry. Within the brain, we hypothesized that enhanced self-referential processing (at baseline in GAD and following the perseverative cognition induction in HC) is associated with increased engagement of the default mode network which in turn accounts for difficulties in focusing on the tracking task (i.e., levels of default mode network de-activation at target appearance). To inform our understanding of HRV as a putative marker for neural processes involved in maladaptive cognition (Thayer, Ahs, Fredrikson, Sollers, & Wager, 2012), we tested if

higher levels of HRV predict better ability to deactivate the default mode network during the transition from internal thoughts to task-focused behaviour.

Methods and Materials

Participants

We recruited 40 individuals to take part in the study, which was approved by the National Research Ethics Service (NRES) for the National Health Service (NHS) with university sponsorship granted via the Brighton and Sussex Medical School Research Governance and Ethics Committee. Participants were compensated for their time. One GAD subject had to be excluded from the analyses because of missing data. The final sample was composed of 19 individuals who met diagnostic criteria for generalized anxiety disorder (GAD; 17 women, 2 men; mean age \pm S.D. = 29.6 ± 6.9 years) and 20 healthy controls (17 women, 3 men; mean age = 28.7 ± 9.5 years). Only 1 participant was non-Caucasian. Patients were recruited from public advertisement and from Access and Recovery Services clinics of Sussex Partnership NHS (Mental Health) Foundation Trust. Healthy control participants were recruited by advertisement from staff and students of the University of Sussex, and Brighton and Sussex Medical School. All participants were right-handed, native English speakers, and had normal or corrected-to-normal vision.

Exclusionary criteria were: age younger than 18 years, prior history of head injury, major medical neurological or psychiatric disorder (other than GAD and co-morbid depression for the patient group), cognitive impairment, history of substance or alcohol abuse or dependence, diagnosis of heart disease, obesity (body mass index $> 30\text{kg/m}^2$), pregnancy, claustrophobia or other general MRI exclusions. The exclusion of co-morbid psychiatric disorders other than depression was due to: a) the knowledge that the psychiatric diagnosis occurring most often in comorbidity with GAD is major depressive disorder (e.g., Beesdo et al., 2010); b) the need to have an –as much as possible- unmedicated sample; c) the focus of the paper on poor inhibition as a key factor in anxiety and the need to exclude confounding variables in drawing our conclusions (i.e.,

our hypotheses were driven by previous data on autonomic and neural correlates of generalized anxiety disorder without considering the effects of other comorbid disorders).

Two GAD participants were included who use long-term medications (1 citalopram, 1 pregabalin) at the time of the study. All other patients and controls were medication free. All participants provided written informed consent.

Procedure

The Structured Clinical Interview for DSM-V (SCID) was administered by a trained postdoctoral fellow (FM) to both patient and controls to confirm/exclude the diagnosis of GAD and comorbid disorders. Participants then completed a series of online socio-demographic and dispositional traits questionnaires. Participants were subsequently familiarized with the neuroimaging environment, connected to the physiological recording equipment, and then underwent the fMRI protocol.

Questionnaires

All participants completed a set of questionnaires accessing socio-demographic, and lifestyle (nicotine, alcohol, and caffeine consumption, physical activity) information.

Dispositional perseverative cognition was assessed by:

1) Stress-Reactive Rumination Scale (SRRS; Robinson & Alloy, 2003), a measure of the tendency to engage in perseverative cognition after stressful events. The scale was designed to measure rumination in a manner that is not confounded with depressive symptoms, a limitation of many other self-report perseverative cognition scales (Robinson & Alloy, 2003). The scale has the following subscales: Negative Inferential Style, Hopelessness, and Active Problem-Solving.

2) Penn State Worry Questionnaire (PSWQ; Meyer et al., 1990), a 16-item self-report questionnaire commonly used to measure pathological worry in both clinical and non-clinical populations. Respondents report how true each statement is for them on a 5-point Likert scale (1 =

not at all typical of me, 5 = *very typical of me*). The PSWQ has shown good-to-excellent internal consistency (α s = .86 - .93 across clinical and college samples; Molina & Borkovec, 1994) and good discriminant validity (i.e., discriminates GAD from other anxiety disorders; Brown, Antony, & Barlow, 1992).

To better characterize the sample from a clinical point of view, levels of state and trait anxiety (State-Trait Anxiety Inventory, STAI; Spielberger et al., 1983) and depression (Beck Depression Inventory, BDI; Beck et al., 1988) were also assessed (see Makovac et al., 2015 for further details).

Experimental Task and Design

While in the scanner, participants performed a series of three 6-min easy visuomotor tracking tasks interspersed by four 5-min resting state periods. The first and last runs were resting state runs. There were no time intervals between subsequent runs, and the next run began as soon as the participant completed the visuo-analogue scales (VASSs; see the section below) at the end of the preceding run.

The tracking task (adapted from Ottaviani et al., 2013) required participants to follow a white circle moving slowly and horizontally on the screen and to press a button as fast as possible each time the circle turned red. The duration of the red circle (target) was 100 ms after which the circle returned to white and the task continued. For each target, accuracy and reaction times were recorded. The circle made six transits of about a minute each. Targets were presented at random intervals: there were a total of 9 targets with a maximum of 2 targets (at least 20 sec apart) per transit. The level of difficulty was very low, to increase the likelihood of episodes of perseverative cognition. Randomly, prior to either the second or third performance of the tracking task participants underwent a verbal induction procedure designed to engender perseverative negative cognition. Participants were randomised according to when the induction occurred to permit control of temporal order effects. The induction took the form of a recorded verbal instruction delivered to the participant as follows:

“Next I would like you to recall an episode that happened in the past year that made you feel sad, anxious, or stressed, or something that may happen in the future that worries you. Then, I would like you to think about this episode in detail, for example about its possible causes, consequences, and your feelings about it. Please keep thinking about this until the end of the next tracking task. Thank you. Please take as much time as you need to recall the episode and press the button whenever you are ready”.

This type of induction has been widely used and proved to be effective in eliciting perseverative cognition in healthy and pathological subjects (Ottaviani et al., 2016 for a meta-analysis).

Visual analogue scales (VAS)

After each tracking task (and resting state run), to assess state levels of perseverative cognition over the preceding period, participants were asked about their thoughts, using VASs.

The participants were asked to rate on separate 100-point VASs “how much, for the duration of the task, they were”: 1) focused on the task?; 2) distracted by external stimuli?; 3) ruminating/worrying?; and 4) distracted by internal thoughts?.

Physiological data processing

Heartbeats were monitored using MRI-compatible finger pulse oximetry (8600FO; Nonin Medical). The analog physiological signals were digitized at 1000 Hz using a CED 1401 analog-to-digital converter (Cambridge Electronic Design) and analysed using Spike 2 software (Cambridge Electronic Design). Each signal epoch was manually checked and corrected for artefacts. As the root mean square successive difference (RMSSD) has been demonstrated to be a reliable parameter for assessing vagus-mediated HRV from very short (down to 10 s) heart rate recordings (e.g., Nussinovitch et al., 2011), HR and RMSSD were obtained for each task epoch using RHRV 4.0 (<http://rhrv.r-forge.r-project.org>). As recommended, HRV data was converted to natural logarithms

(lnHRV).

MRI acquisition and image pre-processing

All images were acquired on a 1.5-Tesla Siemens Magnetom Avanto scanner. Blood oxygenation level dependent (BOLD) signals were recorded using spin echo sequence, 34 slices, slice thickness 3 mm, Inter-slice gap 0.6 mm, voxel size 3 x 3 x 3 mm, TR 2520 ms, TE 43 ms.

For the fMRI pre-processing the first four volumes were discarded to allow the stabilisation of longitudinal magnetization. Pre-processing was conducted using FSL (FMRIB Software Library, www.fmrib.ox.ac.uk/fsl). Motion correction was conducted using FMRIB's Linear Registration Tool (MCFLIRT; Jenkinson, Bannister, Brady, & Smith, 2002; Jenkinson & Smith, 2001). Slice timing correction, spatial smoothing with a Gaussian kernel of 8 mm (FWHM), and high pass temporal filtering (cut-off full-width 100 sec) to remove low-frequency drift were applied. The EPI data was then registered to the individual's high-resolution structural scan using the Boundary-Based Registration (BBR) algorithm. Registration to Montreal Neurological Institute (MNI) standard space was then implemented using FNIRT (FMRIB's non-linear registration tool) nonlinear registration.

Statistical Analyses

Questionnaire, behavioural, and HRV analyses

All data are expressed as means \pm SD. Differences at $p \leq 0.05$ are regarded as significant. Data analyses were performed with SPSS 22.0 for Windows (SPSS Inc., USA).

Analyses on pre-existing group differences and the effects of the induction at a behavioural (RTs), subjective (VAS), and physiological (RMSSD) level are described elsewhere, although on a slightly different sample size (Makovac et al., 2015). For clarity purposes, however, the main findings will be summarized in the Results section.

To examine the role of HRV as a marker for cognitive rigidity, Pearson correlations were

performed between baseline HRV and change scores in perseverative cognition from pre- to post-induction.

fMRI data analysis

All 1st Level and Higher Level analyses were performed using FEAT Version 6.0, part of FSL (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl).

First-level analyses

BOLD response was modelled using two explanatory variables; the first represented a 15 s pre-target period (proxy of internally-oriented attention) and the second a 15 s post-target period (proxy of task-oriented attention). We opted for 15 s in light of past evidence showing that the mean duration of each mind-wandering episode is about 14 s (Klinger, 1978). Our approach is consistent with previous work that, despite the lack of knowledge as to how long participants had actually been in a particular mind-wandering state, based the analyses on the assumption that the 15 s prior to each target/probe would consistently fall within that mind-wandering state on average (e.g., He et al., 2011; Smallwood et al., 2008). The appearance of infrequent targets is supposed to force participants to go back on task whereas, given the monotony of the task and the infrequency of target appearance, periods immediately prior to the latter are likely to be characterized by internally-oriented attention (e.g., Smallwood, 2011). The tracking task used in the present study has proven to be effective in eliciting episodes of mind wandering (before induction) and perseverative cognition (after induction) in healthy individuals (Ottaviani et al., 2013).

These were used for the 1st Level FEAT analysis. For each explanatory variable, the regressor was convolved with a gamma function to produce an expected BOLD response. The temporal derivative of this time-course was also included in the analytic model for each variable to capture temporal shifts. Two contrasts were tested: pre-target activation greater than post-target activation ($\text{Pre}_{\text{tgt}} > \text{Post}_{\text{tgt}}$) and pre-target activation less than post-target activation ($\text{Pre}_{\text{tgt}} < \text{Post}_{\text{tgt}}$).

Second-level analyses

After analysing the tracking tasks before and after the induction for each subject, data were passed into a higher-level analysis, which allowed within- and between-groups comparisons. The interspersed resting-state periods go beyond the focus of the present work and will be excluded from the analyses. Higher-level analyses were carried out using FLAME (FMRIB's Local Analysis of Mixed Effects), such that group-level effects were modelled using random effects. Z-statistics images were threshold based upon clusters determined by $z > 2.3$ and a (corrected) cluster significance threshold of $p < 0.05$. Models were created to examine the following properties:

1. Main effect of GROUP (GAD vs HC);
2. Main effect of INDUCTION (Before vs After);
3. GROUP x INDUCTION interaction.

For visualization purposes, the coefficients of parameter estimates for regions that showed significant activation differences in these contrasts were extracted and plotted.

In addition to the above analyses, to explore individual differences in cognitive functioning and brain activation, specific correlational analyses were undertaken. These tested for association between key physiological and behavioural measures and neural activity. Variables of interest were SRRS (tendency to engage in perseverative cognition) and HRV. These were modelled as explanatory variables in whole brain regression analyses. Separate analyses were performed for pre- and post-induction blocks for HRV.

Outliers were controlled for, using automatic outlier de-weighting in FSL. This tool automatically detects outlier data points (for each voxel, each participant's data is considered with respect to the others to determine if it is an outlier). Outliers are then automatically de-weighted in the multi-participant statistics. All fMRI data shown were cluster-corrected for multiple comparison at $z > 2.3$, $p < 0.05$. For visualization purposes, coefficients of parameter estimates for

regions that showed significant correlations with the physiological and behavioural variables were extracted and plotted for these measures.

Results

Descriptive results

No group differences for any of the assessed socio-demographic and lifestyle variables emerged, therefore these were not included as covariates in the subsequent analyses (see Makovac et al., 2015). GAD participants had higher (and pathological) scores on the PSWQ (69.1 ± 8.2 vs 42.6 ± 13.4 , $t(38) = 7.02$, $p < 0.001$) and the SRRS (1398 ± 263 vs 983 ± 238), $t(38) = 4.92$, $p < 0.001$) compared to controls. Levels of trait anxiety were significantly higher in the GAD group (55.2 ± 8.8 vs 35.8 ± 9.4 , $t(38) = 7.01$, $p < 0.001$) compared to controls. According to Beck & Steer (1984) cut-offs, GAD participants reported mild-to-moderate levels of depression (16.1 ± 10.2), whereas the control group was in the no-depression range (4.1 ± 4.9), $t(38) = 4.81$, $p < 0.001$.

Induction effect at a behavioural, cognitive and physiological level

After the perseverative cognition induction, all participants were less focused on task ($F(1,38) = 22.2$; $p < .0001$), more distracted by internal thought ($F(1,38) = 30.44$; $p < .0001$) and less distracted by external stimuli ($F(1,38) = 8.46$; $p = .01$), engaged more in rumination and worry ($F(1,38) = 82.02$; $p < .0001$), and had an overall increase in reaction times ($F(1,38) = 21.14$; $p < .0001$) suggesting difficulties in disengaging thoughts. The task was too easy to allow for accuracy analysis. Particularly, GAD patients had a stronger loss of attentional focus compared to controls as shown by the Induction X Group interaction ($F(1,38) = 6.32$; $p < .0001$) and significant post hoc comparisons ($t(18) = 5.60$; $p < .0001$). Moreover, GAD patients had an overall lower HRV than healthy participants, as indicated by the main effect of Group in this analysis ($F(1,38) = 11.43$; $p = .002$).

HRV as a marker for cognitive rigidity

Significant correlations were found between baseline (pre-induction) HRV ($61.4 \pm 38.9 \text{ ms}^2$) and pre- to post-induction change scores in levels of perseverative cognition (assessed by the VAS “ruminating/worrying?”; $\Delta = 40.3 \pm 38.5$), suggesting a higher increase in perseverative cognition in participants with lower HRV ($r = -0.33$; $p = 0.04$).

Neurobiological substrates of the transition from spontaneous self-generated thoughts to attentional control

The detection of infrequent targets deactivated brain regions primarily attributed to the default mode network. Across groups, discounting induction effects, target presentation elicited decreases in activation ($\text{Pre}_{\text{tgt}} > \text{Post}_{\text{tgt}}$) in regions of the left precuneus, postcentral gyrus and Heschl’s Gyrus, and, bilaterally, regions of the precentral gyrus, juxtapositional lobule cortex, lateral occipital cortex, and occipital pole (Table 1a, Figure 1A₁). Increased activation following target presentation ($\text{Post}_{\text{tgt}} > \text{Pre}_{\text{tgt}}$) was noted in Crus I and Crus II of the left cerebellum (Table 1b, Figure 1A₂).

Neurobiological substrates of abnormal inhibitory processes following the induction

A significant main effect of Induction and a Group x Induction interaction were shown: Both occurred for the $\text{Pre}_{\text{tgt}} > \text{Post}_{\text{tgt}}$ contrast. No suprathreshold main effect of Group was observed.

The induction altered neural responses from pre- to post-target appearance ($\text{Pre}_{\text{tgt}} > \text{Post}_{\text{tgt}}$) in areas of the frontal pole (bilaterally), superior frontal gyrus (right), and paracingulate gyrus (right) (see Table 2, Figure 1B). Examination of the coefficient of parameter estimate (COPE) values from a regional mask encompassing these regions showed that target appearance evoked a greater reduction in activation post induction, compared to pre induction (Figure 1B₁).

A Group x Induction interaction ($\text{Pre}_{\text{tgt}} > \text{Post}_{\text{tgt}}$) was evident within precuneus (bilateral), lateral occipital gyrus (right), and posterior cingulate gyrus (bilateral) (see Table 3). Examination

of the COPE estimates for a regional mask based on this interaction indicated that, after the induction, the activation patterns of the two groups tended to converge. The significant interaction was driven by a stronger reduction of activation in healthy individuals after target presentation from pre- to post-induction while, in the GAD group, this reduction was already evident before induction (Figure 1C).

Additional analysis of target occurrence

To confirm that the reported findings reflected the properties of transition between attentional states rather than target detection, a follow-up event-related analysis using the target presentation times as the events and event length as length of target display (i.e. 500 msec) was conducted. Pre-induction, there was a main effect of group, the HC group displayed greater activation than GAD in a cluster located in the right frontal pole ($z_{\max} = 3.96$, MNI coordinates 26,56,6; $z > 2.3$, cluster corrected $p < 0.05$; Online Supplement Table 1). This effect was absent in the post-induction analysis. Neither a main effect of Induction nor a Group x Induction interaction emerged from this analysis further supporting the view that the above results do not reflect target detection per se (Online Supplement Fig. 1).

Individual differences in trait perseverative cognition and inhibitory processes

Correlational analyses were undertaken to examine relationships between neural responses from pre- to post-target appearance and SRRS. Both contrasts ($\text{Pre}_{\text{tgt}} > \text{Post}_{\text{tgt}}$ and $\text{Post}_{\text{tgt}} > \text{Pre}_{\text{tgt}}$) were tested. In both groups, scores on the SRRS were significantly associated with the shift in activation following the induction (Figure 1B₂). More precisely, after the induction participants with higher dispositional tendencies to engage in perseverative cognition showed less deactivation within frontal pole regions at target presentation compared to low-trait SRRS individuals suggesting difficulties in disengaging thoughts.

Individual differences in HRV and inhibitory processes

Individual differences in HRV predicted the capacity for neural activity to shift from an internally-directed pattern supporting perseverative negative thoughts, to activity associated with control of externally-directed attention. HRV correlated negatively with both pre- and post-induction decreases in neural responses evoked by target presentation ($\text{Pre}_{\text{tgt}} > \text{Post}_{\text{tgt}}$). In other words, as HRV decreased, the magnitude of activation changes brought about by target detection increased. Before induction, HRV correlated with target-induced deactivation within right superior parietal lobule, supramarginal gyrus and superior frontal gyrus (Table 4a, Figure 2a). Following the induction, HRV negatively correlated with target-induced decreased activity bilaterally within central and parietal opercular cortices and precentral and postcentral gyri (Table 4b, Figure 2b).

HRV did not correlate with target-related changes in activity before induction. However, following the induction, a negative correlation was noted for regions that encompassed right middle and inferior temporal gyri and Crus I and Crus II regions of the cerebellum: As HRV increased, there was less activation in these regions from pre- to post-target appearance (Figure 2c).

Discussion

Combining neuroimaging techniques with peripheral physiology monitoring provides insight into brain-body interactions and the integration of autonomic, affective, and cognitive processes (Critchley, 2009). To our knowledge, this is the first functional imaging study in which simultaneous neuroimaging and autonomic recording were used to investigate difficulties in inhibiting perseverative thoughts and autonomic arousal, the most pervasive symptoms of GAD. We first examined the brain areas associated with the transition from spontaneous self-generated thoughts to attentional control during a low-demanding task in GAD and healthy participants. Second, we studied the consequences of a perseverative cognition induction on this shift from internally- to externally-oriented attention. Then, we tested if the effects of the induction on this attentional shift could be predicted by dispositional tendencies to engage in perseverative cognition

(scores on the SRRS). Lastly, we examined the association between HRV and brain activation changes during the transition from internal thoughts to task focusing.

The neural responses elicited by target appearance were principally expressed as a relative deactivation rather than activation within specific brain regions. This was particularly the case for regions which form part of the default mode network, including the precuneus, which is implicated in episodic memory, visuospatial processing, self-reflection and aspects of consciousness (Fox, Spreng, Ellamil, Andrews-Hanna, & Christoff, 2015; Hannawi, Lindquist, Caffo, Sair, & Stevens, 2015; Kwok & Macaluso, 2015). Target appearance also attenuated activity within motor, auditory, and somatosensory areas, putatively reflecting the rich multisensory nature of internal thoughts (e.g., Fox, Nijeboer, Solomonova, Domhoff, & Christoff, 2013; Hasenkamp et al., 2012). Perhaps surprisingly, we did not find target-related changes within default mode network regions, notably posterior cingulate cortex, which is also linked to self-referential thoughts and worry (e.g., Christoff, Gordon, Smallwood, Smith, & Schooler, 2009; Paulesu et al., 2010). Present results are, however, consistent with a recent meta-analysis of neuroimaging studies, which concluded that default mode activation alone is insufficient to capture fully the neural basis of spontaneous thought (Fox et al., 2015). Also, in the present study we sought to address the concept of cognitive “flexibility” by specifically targeting transitions from internal thoughts to externally oriented attention, and therefore focused on a different research question compared to studies directly comparing brain correlates of worrisome and neutral thoughts (e.g., Hoehn-Saric et al., 2004; Paulesu et al., 2010).

We replicated the effects of a perseverative cognition induction (e.g., Ottaviani, Shapiro, & Fitzgerald, 2011; Ottaviani & Shapiro, 2011) on target detection performance, showing decreased focus on the task and slower reaction times to target appearance indicative of difficulties in getting out of a state of heightened focus on ruminative and worrisome thoughts (Franklin, Smallwood, & Schooler, 2011; Ottaviani et al., 2013). Moreover, baseline HRV was associated with pre-to post-induction changes in perseverative cognition, confirming the previously reported association

between cognitive inflexibility and autonomic rigidity (Ottaviani et al., 2013; 2015a,b).

In line with the notion that worry in GAD is likely to be more quantitatively than qualitatively different from normal worry (Borkovec, Alcaine, & Behar, 2004; Ruscio & Borkovec, 2004), the induction increased the amount of perseverative negative thoughts in controls up to an equivalent level reported by GAD patients, making the two groups more similar.

Our implementation of an induction procedure extended what has been previously observed on changes in brain activity: an earlier study reported that worry-inducing sentences, enhances the activity within medial prefrontal cortices when compared to neutral sentences in both GAD patients and controls (Paulesu et al., 2010). We observed an increase in activity after induction within frontal pole, superior frontal gyrus, and paracingulate gyrus, regions implicated in self-referential processing and introspection. These regions decreased their responses to target appearance suggesting a role in self-related mental imagery. Induction amplified the pre-target engagement of these regions in perseverative cognition, particularly in healthy controls since it is likely that in GAD patients this effect was already present before the induction. Thus, the induction had the effect to make the two groups more similar both at a behavioural and at a neural level. These findings extend to anxiety disorder previous observations from pathological worriers (Ottaviani et al., 2014) and patients with major depression (Ottaviani et al., 2015b).

A dimensional approach to psychopathological symptoms was further supported by the finding that scores on a continuous measure of dispositional perseverative cognition (SRRS) also predicted these effects of induction on prefrontal areas activation. High trait participants were characterized by less prefrontal deactivation at target appearance after the induction compared to low trait participants. Though focusing on worry, a similar relationship has been found between scores on the PSWQ and sustained activation of the medial frontal region after worry-induction (Paulesu et al., 2010). Together, these findings converge to suggest that these brain areas mediate the link between perseverative cognition and both the anticipation of threat and the lack of recovery from threat.

Participants with lower pre-induction HRV were characterized by stronger activation changes in areas such as the superior parietal lobule, supramarginal gyrus, and superior frontal gyrus, all critically important for the manipulation of information in working memory (du Boisgueheneuc et al., 2006; Koenigs, Barbey, Postle, & Grafman, 2009), spontaneous thought processes (Fox et al., 2015), and the dynamic switch between external and internal modes of attention (Andrews-Hanna, Smallwood, & Spreng, 2014). This is a counterintuitive result, as one may expect higher (and not lower) HRV to index a better ability to switch from internally-oriented to externally-oriented attention, and hence be associated with stronger deactivation in such areas. Nevertheless, participants with lower HRV (GAD patients in our sample) are likely to engage in more perseverative cognition compared to those with higher HRV. Thus, larger changes (i.e., stronger deactivation of these areas at target appearance) would be entirely due to baseline differences. This is supported by the significant correlation between baseline HRV and pre-to post-induction changes in perseverative cognition, consistent with established relationships between HRV and performance on cognitive tasks under threat conditions (Hansen, Johnsen, & Thayer, 2003, Hansen, Johnsen, & Thayer, 2009).

Interestingly, pre- and post-induction associations implicated different regions, suggesting that HRV is able to predict differences in brain activity during both “normal” mind wandering and dysfunctional perseverative cognition. Whereas before the induction, HRV was associated with activity principally within working memory areas, after the induction lower levels of HRV were correlated with a stronger deactivation of somatosensory areas (postcentral gyrus, parietal operculum), reflecting the idea that perseverative cognition is akin to being more immersed in imagined situations. Future fMRI studies directly comparing functional and dysfunctional forms of repetitive thinking are needed to clarify if mind wandering and perseverative cognition can be differentiated by the degree to which they recruit attentional or salience-related brain networks, respectively.

When we looked at post-induction activation patterns, participants with lower HRV levels

expressed higher activation within the cerebellum and the inferior temporal gyri. The cerebellum is certainly involved in physiological regulation as a component of the central autonomic network (Spyer, 1999) contributing to the control of cardiovascular responses through its connections with the hypothalamus (Critchley, Corfield, Chandler, Mathias, & Dolan, 2000). Accordingly, covariation is observed between HRV and the cerebellum (Critchley et al. 2003; Gianaros, Van Der Veen, & Jennings, 2004; Lane et al., 2009; Napadow et al., 2008).

The temporal lobe regions are associated with counterfactual reasoning (Van Hoeck et al., 2014), including retrieving, reliving and elaborating past events (conscious episodic memory) (Andreasen et al., 1999; Addis, Wong, & Schacter, 2007) and imagining potential futures (Summerfield, Hassabis, & Maguire, 2010; Weiler, Suchan, & Daum, 2010). Our data raise the intriguing notion that there may be physiological constraints on counterfactual thought. Low tonic levels of HRV might indicate a predisposition to keep on ‘erring on the side of caution’ (Gerin et al., 2013) reflected in perseverative thinking on the psychological level. Here, we provided the first evidence for brain areas mediating this relationship by bridging the central autonomic and the cingulo-opercular networks.

Our findings add to knowledge regarding HRV as a potential marker of stress and health (Thayer et al., 2012), shedding light into the still unexplained relationship between anxiety disorders and cardiovascular risk (see Player and Peterson, 2011 for a review). Considering that perseverative cognition is one of the most difficult symptoms to treat in anxiety, our results may also have important implications for therapy. HRV is a significant predictor of treatment response and remission from major depression (Chambers and Allen, 2002; Jain et al., 2014), and HRV biofeedback is a promising tool for the treatment of mood disorder (Karavidas et al., 2007). Our results argue for the extension of these pilot intervention studies to anxiety disorders. Moreover, in a research domain, our results suggest HRV is a useful tool to detect and index perseverative cognition without interrupting or interfering with free thinking. Given that spontaneity is a key feature of intrusive thoughts, future investigations may increase ecological validity by triggering

thought probes based on the physiological (i.e., HRV) changes of study participants.

We acknowledge that the statistical power of our study will likely improve with a larger sample size. A second limitation is that the occurrence of perseverative cognition while participants are not focusing on a task could only be assumed. To reduce this bias, we chose an induction manipulation that has been extensively proven to be effective in eliciting worrisome and ruminative thoughts. Moreover, an increase in these types of thoughts from pre- to post-induction was testified by self-report measures (VAS). Limitations notwithstanding, our findings highlight the dynamic relationship between mind and body by identifying neural substrates that shape aberrant cognitions in anxiety through psychophysiological interaction with autonomic bodily state. An integrative investigative approach is called for to better inform therapeutic interventions, combining techniques including functional brain imaging and detailed autonomic monitoring, and drawing on both basic research and clinical observations.

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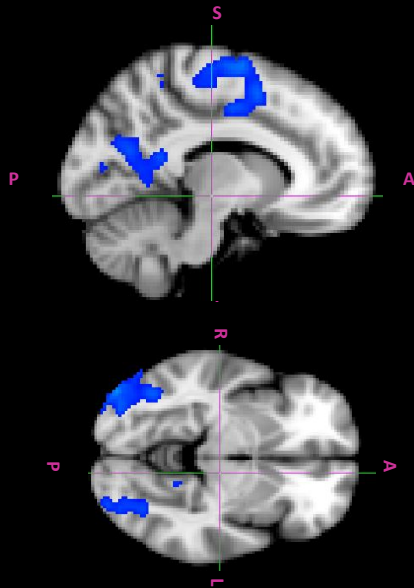
Figure captions

Figure 1. A1. Brain regions exhibiting reductions in BOLD signal from pre- to post-target appearance ($\text{Pre}_{\text{tgt}} > \text{Post}_{\text{tgt}}$; $z < 2.3$, cluster corrected $p < 0.05$) across groups. A2. Brain regions exhibiting increases in BOLD signal from pre- to post-target appearance ($\text{Post}_{\text{tgt}} > \text{Pre}_{\text{tgt}}$; $z < 2.3$, cluster corrected $p < 0.05$) across groups. B. Brain regions showing a main effect of Induction ($\text{Pre}_{\text{tgt}} > \text{Post}_{\text{tgt}}$; $z > 2.3$, cluster corrected $p < 0.05$). B1. Main effect of Induction (median COPE value within activation mask). B2. Correlation between contrast estimates in prefrontal area and trait rumination (SRRS scores). C. Brain regions showing significant Group x Induction effect on BOLD activation signal ($\text{Pre}_{\text{tgt}} > \text{Post}_{\text{tgt}}$; $z < 2.3$, cluster corrected at $p < 0.05$). Group by Induction Interaction Effect: Cope estimates inside activation mask (median values). The embedded graph shows the Group by Induction Interaction Effect (median COPE value within activation mask).

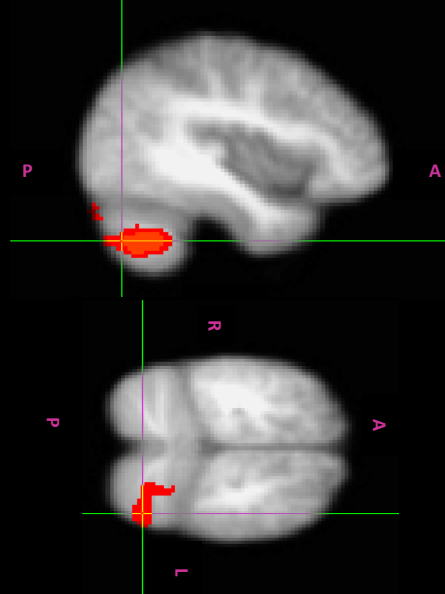
Figure 2. Pre- (A) and post-induction (B) correlations between changes in BOLD signal from pre- to post-target appearance ($\text{Pre}_{\text{tgt}} > \text{Post}_{\text{tgt}}$) and HRV. C. Correlation between change in BOLD signal from pre- to post-target appearance ($\text{Pre}_{\text{tgt}} < \text{Post}_{\text{tgt}}$) and HRV. Embedded graphs show the relationships between brain region activation changes at target appearance (median COPE value within activation mask) and $\ln(\text{HRV})$.

A. Change in BOLD signal after target detection

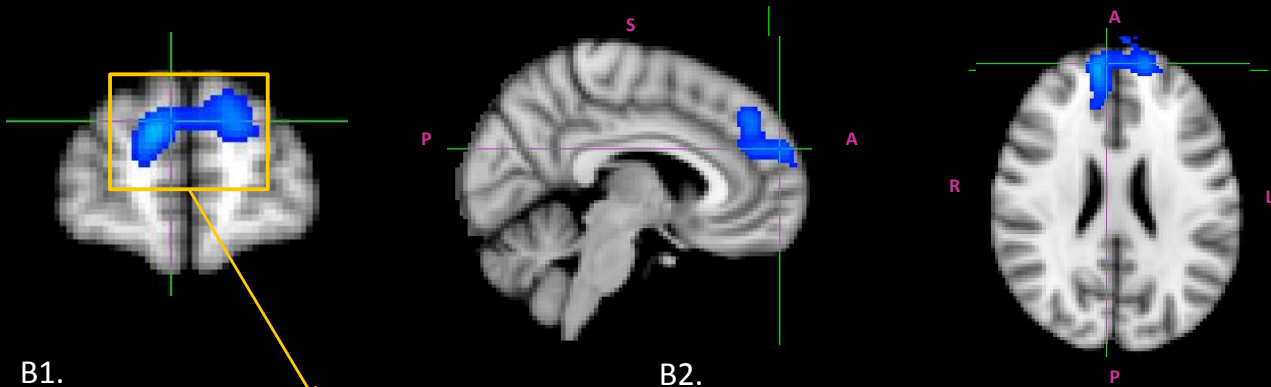
A1. $\text{Pre}_{\text{tgt}} > \text{Post}_{\text{tgt}}$



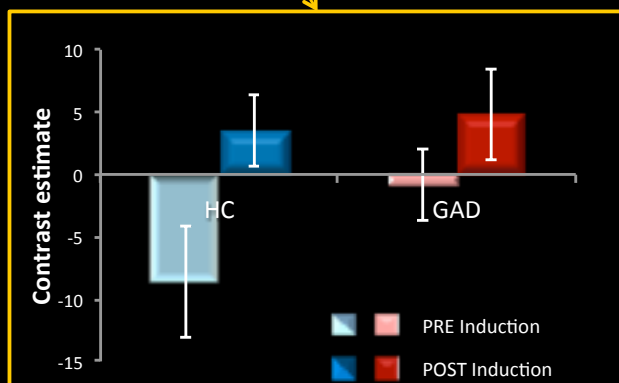
A2. $\text{Pre}_{\text{tgt}} < \text{Post}_{\text{tgt}}$



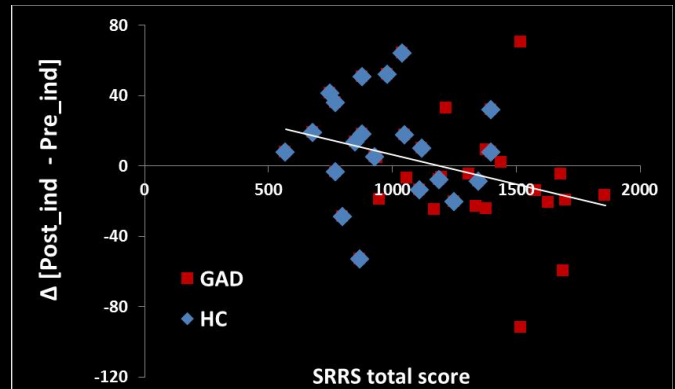
B. Main effect of Induction



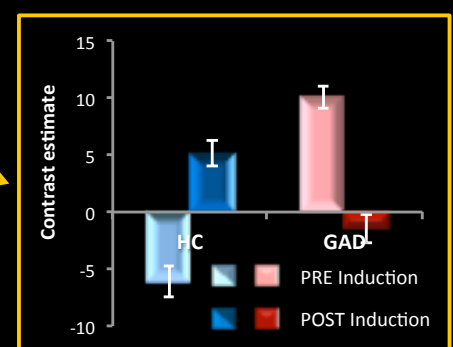
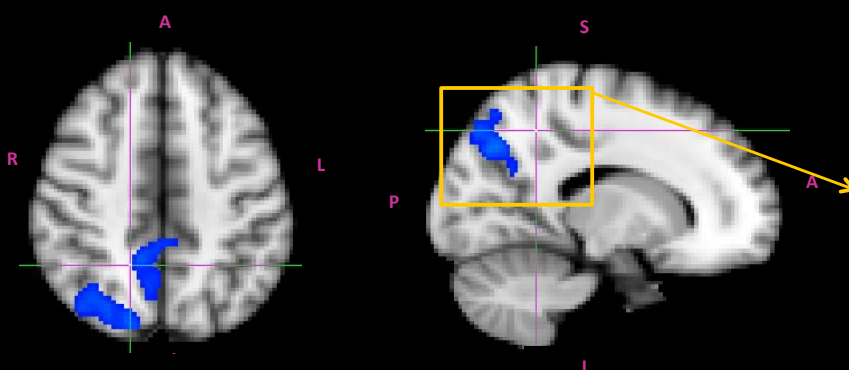
B1.



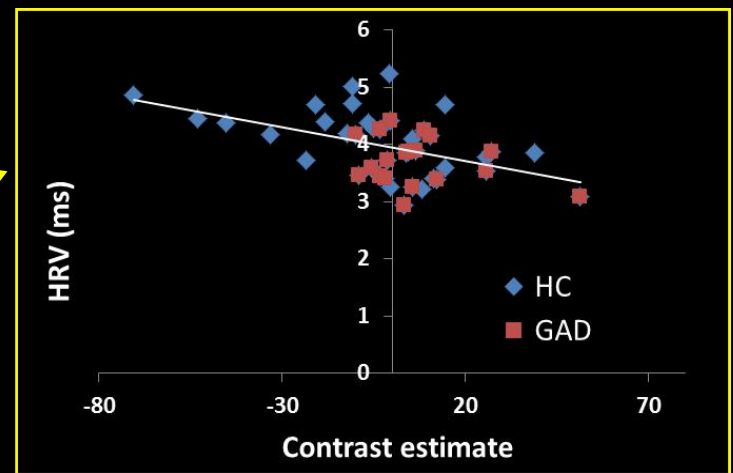
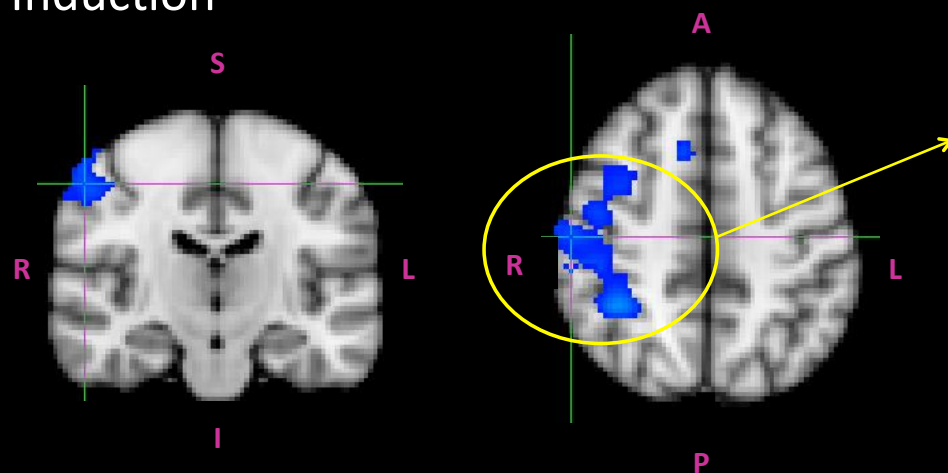
B2.



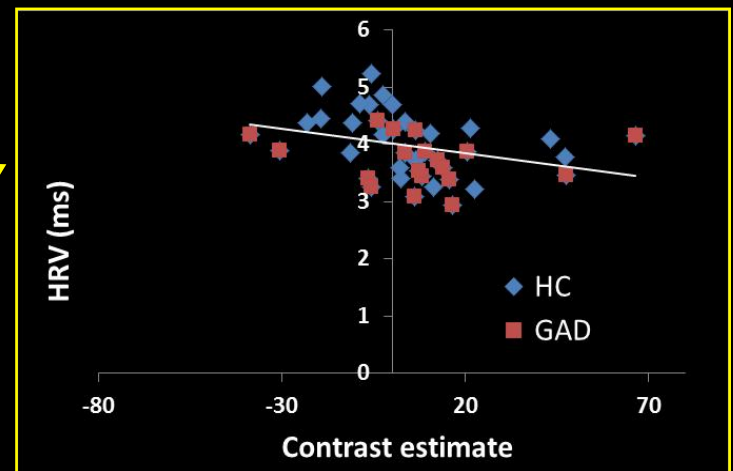
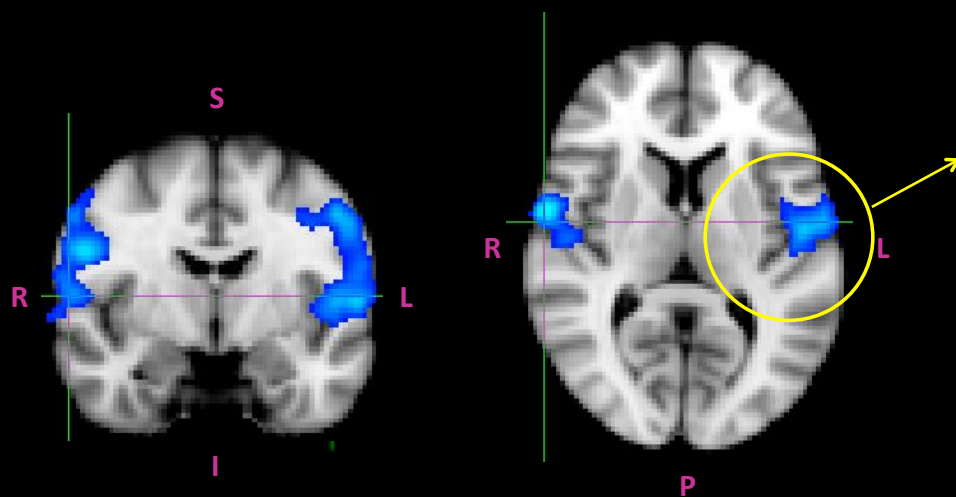
C. Group x Induction interaction



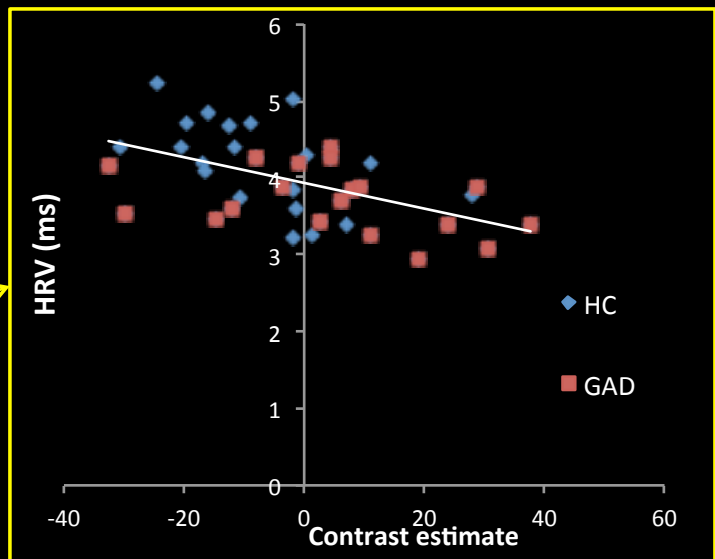
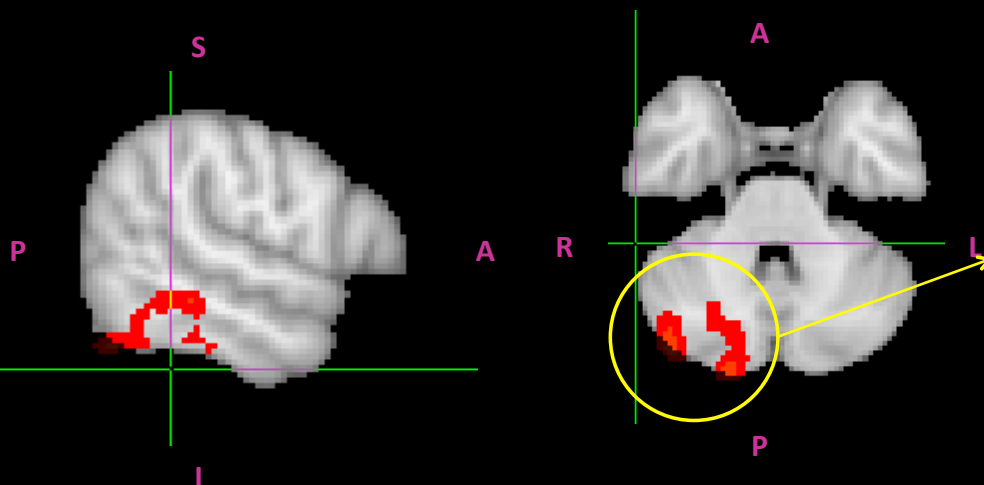
A. Correlation with HRV – [$\text{Pre}_{\text{tgt}} > \text{Post}_{\text{tgt}}$] Pre Induction



B. Correlation with HRV – [$\text{Pre}_{\text{tgt}} > \text{Post}_{\text{tgt}}$] Post Induction



C. Correlation with HRV – [$\text{Pre}_{\text{tgt}} < \text{Post}_{\text{tgt}}$] Post Induction



Tables

Table 1. Maxima of regions showing a significant ($z > 2.3$, cluster corrected at $p < 0.05$) main effect of Target for $\text{Pre}_{\text{tgt}} > \text{Post}_{\text{tgt}}$ (a) and $\text{Post}_{\text{tgt}} > \text{Pre}_{\text{tgt}}$ (b) contrasts across groups.

Contrast	Z	x	y	z	Hemisphere	Region of activation
(a) $\text{Pre}_{\text{tgt}} > \text{Post}_{\text{tgt}}$	4.27	-38	-22	52	Left	PreCentral Gyrus
	3.9	10	-12	62	Right	Juxtapositional Lobule Cortex
	3.44	12	-16	72	Right	PreCentral Gyrus
	3.42	-8	-4	66	Left	Juxtapositional Lobule Cortex
	3.35	-36	-12	58	Left	PreCentral Gyrus
	3.3	-18	-14	58	Left	PreCentral Gyrus
	3.52	-38	-70	4	Left	Lateral Occipital Cortex
	3.01	-42	-28	22	Left	Parietal Operculum Cortex
	2.99	-12	-50	16	Left	Precuneus
	2.97	-38	-28	22	Left	Parietal Operculum Cortex
	2.95	-10	-66	20	Left	Precuneus
	2.88	-30	-94	-6	Left	Occipital Pole
	3.93	44	-88	-4	Right	Lateral Occipital Cortex
	3.69	36	-96	10	Right	Occipital Pole
	3.46	40	-90	6	Right	Occipital Pole
	3.31	32	-84	-2	Right	Lateral Occipital Cortex
	3.13	42	-62	-8	Right	Lateral Occipital Cortex
	3.04	44	-68	-4	Right	Lateral Occipital Cortex
(b) $\text{Post}_{\text{tgt}} > \text{Pre}_{\text{tgt}}$	4.02	-46	-56	-38	Left Cerebellum	Crus I
	3.89	-40	-66	-44	Left Cerebellum	Crus II

3.38	-12	-90	-24	Left Cerebellum	Crus I
3.17	-34	-82	-28	Left Cerebellum	Crus I
3.12	-16	-86	-22	Left Cerebellum	Crus I
3.1	-20	-80	-22	Left Cerebellum	Crus I

Table 2. Maxima of regions showing a significant ($z > 2.3$, cluster corrected at $p < 0.05$) main effect of Induction (before vs after) across groups.

Z	x	y	z	Hemisphere	Region of activation
4.22	12	56	22	Right	Frontal Pole
3.56	-14	56	26	Left	Frontal Pole
3.55	8	42	38	Right	Superior Frontal Gyrus
3.51	8	44	34	Right	Superior Frontal Gyrus
3.06	18	28	34	Right	Superior Frontal Gyrus
3	18	18	46	Right	Superior Frontal Gyrus

Table 3. Maxima of regions showing a significant ($z > 2.3$, cluster corrected at $p < 0.05$) Group (GAD vs HC) by Induction (before vs after) interaction.

Z	x	y	z	Hemisphere	Region of activation
3.71	30	-72	52	Right	Lateral Occipital Gyrus
3.65	-6	-54	60	Left	Precuneus
3.36	36	-66	52	Right	Lateral Occipital Gyrus
3.33	12	-74	36	Right	Precuneus
3.3	16	-70	38	Right	Precuneus
3.22	36	-62	54	Right	Lateral Occipital Gyrus

Table 4. Maxima of regions showing a significant negative correlation ($\text{Pre}_{\text{tgt}} > \text{Post}_{\text{tgt}}$) with $\ln(\text{HRV})$ pre and post induction.

Contrast	Z	x	y	z	Hemisphere	Region of activation
(a) $\text{Pre}_{\text{tgt}} > \text{Post}_{\text{tgt}}$ pre-induction	3.89	44	-46	62	Right	Superior Parietal Lobule
	3.74	38	-46	50	Right	Superior Parietal Lobule
	3.72	34	-10	38	Right	Precentral Gyrus
	3.22	56	-34	60	Right	Supramarginal Gyrus
	3.18	18	2	60	Right	Superior Frontal Gyrus
	3.11	52	-34	58	Right	Supramarginal Gyrus
(b) $\text{Pre}_{\text{tgt}} > \text{Post}_{\text{tgt}}$ post-induction	4.41	-58	-6	6	Left	Central Opercular Cortex
	4.34	-52	-8	8	Left	Central Opercular Cortex
	4.27	-50	-6	42	Left	Precentral Gyrus
	4.12	-38	-20	50	Left	Precentral Gyrus
	3.49	-60	-8	16	Left	Postcentral Gyrus
	3.46	-42	-14	30	Left	Postcentral Gyrus
	5.06	62	0	8	Right	Central Opercular Cortex
	4.62	56	-6	26	Right	Postcentral Gyrus
	4.08	56	-32	24	Right	Parietal Opercular Cortex
	3.62	64	-16	20	Right	Postcentral Gyrus
	3.57	58	-2	40	Right	Precentral Gyrus
	3.55	72	-18	16	Right	Postcentral Gyrus